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LIST OF SYMBOLS

Symbol		Units
k_M	- Diffusive permeability	$m\ s^{-1}$
D_W	- Diffusion coefficient	$m^2\ s^{-1}$
S_D	- Diffusion pore inlet steric hindrance factor	-
A_k	- Membrane surface porosity	m^2
ΔX	- Membrane thickness	m
L_P	- Hydraulic permeability	$ml\ m^{-2}\ s^{-1}\ atm$
r_P	- Membrane pore radius	m
σ	- Staverman reflection	-
S_F	- Filtration pore inlet steric hindrance factor	-
q	- Solute radius to pore radius factor	-
$f(q)$	- Diffusion friction coefficient	-
$g(q)$	- Filtration friction coefficient	-
J_B	- Water flux	$ml\ s^{-1}$
Δp	- Pressure difference	atm
$\Delta \pi$	- Osmotic pressure difference	atm

J_s	- Solute flux	ml s^{-1}
C_t	- Solute concentration at time t	mg ml^{-1}
C_o	- Solute concentration at time 0	mg ml^{-1}
A	- Membrane area	m^2
V	- Reservoir volume	m^3
α	- Slope of the plot of $\ln(C_t/C_o)$ versus time	min^{-1}
K	- Clearance efficiency coefficient	ml min^{-1}

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CHAPTER 4

EFFECT OF THE ACETIC ACID/PEG RATIOS AND DIFFERENT MOLECULAR WEIGHTS PEG

Since the results in the previous study indicated that the ratio of acetic acid/PEG is a significant factor, different types of PEG are used in this stage of the study. Different molecular weight of PEG was added into the dope formulations at different ratios. Several tests were carried out as to validate the dialysis membrane produced. All the tests were conducted according to the parameter and methodology described in Chapter 3, section 3.2 and individual additional testing condition would be discussed as follows.

4.1 Dope Formulations

In this study, six dope formulations were prepared for each of the additives used. The formulations were designed based on the initial experiments, where the ratio of

acetic acid/PEG was varied between 4 and 14. Design points were added in between the points generated by RSM, CCD. However, an additional mid point beyond the ratio of 14 was also added to further investigate its effect. The six dope formulations used for each type of PEG are shown in Table 4.1. Different molecular weight polyethylene glycol, i.e. PEG 200, PEG 400 and PEG 600 were used for each dope formulations to study the effect on the membrane performance. From the previous chapter, the water content was found to be an insignificant factor in the membrane performance, but its existence is important for its hydrophilic effect and to the membrane morphology. Thus, the amount of water was fixed at 10 %wt, base from the best results obtained in the previous chapter and the cellulose acetate content was maintained at 20 %wt.

4.2 Multi Layer Dialysis Membranes

There are several ways to improve the membrane performance for dialysis process such as increasing the flow rate of the dialysate side and the membrane contacting area. Many attempts had been contributed by other authors to improve the membrane performance (Krieter and Canaud, 2003; Ronco *et al.*, 2000; Muller *et al.*,

Table 4.1 : Formulation of six different dope solutions

No.	Ratio acetic acid/PEG	Acetic acid, %wt	Polyethylene glycol, %wt
1	4	56.00	14.00
2	6.5	60.67	9.33
3	9	63.00	7.00
4	11.5	64.40	5.60
5	14	65.33	4.67
6	16.5	66.00	4.00

2001). Several reports revealed that the treatment period can be reduced from 12 hrs/week to 6 hrs/weeks by using a 5 m² dialyzer instead of the normal 1.2 m² dialyzer, and by increasing the blood and dialysate flow rate from 250 mL/min and 400 mL/min to 500 mL/min and 1000 mL/min respectively (Rotellar *et al.*, 1986; Collins *et al.*, 1986). However, these attempts still remain controversial due to the uncertainty in the solutes removal (Stiller and Mann, 1987).

However, in this study, the flow rate of the both reservoirs is limited below 100 mL/min due to the dialysis cell designed. This is to ensure a complete contact between the solutions flow and the dialysis membrane. Therefore, in order to improve the results obtained, a multi layer dialysis membrane system was used. The concept of this fabricated dialysis cell system is to increase the dialysis membrane contact area from 30 cm² to 90 cm². The testing solutions were allowed flow through 3 pieces of dialysis membrane prepared before recycling it back into the reservoir, which means it came contacted with the dialysate (pure water) trice. Figure 4.1 illustrated the concept of the multi layer dialysis membrane system used. The correlation of membrane performance for multi layer system to single layer system was also determined.

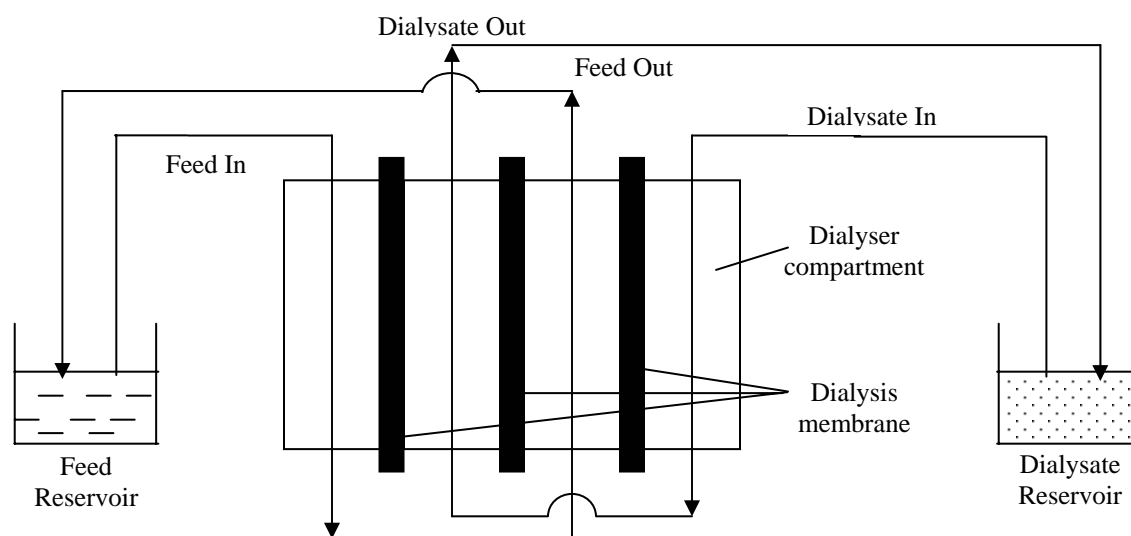


Figure 4.1 Schematic diagram of the concept of multi layer dialysis membrane system

4.3 Membrane Molecular Weight Sieving Efficiency Test

The uremic toxic, which must to be removed from human blood during hemodialysis process are below 200 Daltons (Svartaas *et al.*, 1982) and the molecular weights of proteins, which are considered to cause various chronic side reactions in dialysis patients, are in the range of 10,000 – 55,000 Dalton. However, protein such as albumin must not be diffused out from the human blood during hemodialysis process (Barzin *et al.*, 2004). Therefore, it is important to ensure the molecular weight sieving efficiency of the dialysis membranes produced lies in the acceptable range of dialysis process mentioned. Additionally, this test was also used to differentiate the dialysis membrane produced using different molecular weight PEG as the additives.

The membranes produced from the 3 different formulations were tested using different molecular weight PEG and BSA. The various grades of PEG used for this experiments are PEG 600, PEG 3,000, PEG 10,000 and PEG 35,000 and bovine serum albumin (BSA) 66,000 Dalton that represented the human albumin.

4.3.1 Performance Evaluation Using Polyethylene Glycol (PEG)

The analytical method for determining the concentration of PEG before and after the dialysis process in the testing solution reservoir was proposed by Sabde *et al.* (1997). This method was based on the complex reaction process of the PEG to the reagent used. The analytical reagents prepared were 5 % (w/v) BaCl₂ in 1 N HCl and 2 % (w/v) KI diluted 10 times + 1.27 g I₂.

1 mL reagent BaCl₂ were dispensed into 4 mL sample collected in tubes and 1 mL reagent KI was rapidly added into the tubes and well mixed. Color was allowed to develop for 15 minutes at room temperature. The absorbance of each solution was measured against a blank at wavelength of 535 nm using UV spectrophotometer (UV-Spec Shimadzu UV-160). Equations below were used to determine the PEG concentration and clearance efficiency of each polyethylene glycol used.

$$\text{Sample (mg/mL)} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \text{Standard Concentration (mg / mL)} \quad (4.1)$$

$$\text{PEG clearance} = \frac{\text{Concentration at time } t - \text{Concentration at time } 0}{\text{Concentration at time } 0} \times 100\% \quad (4.2)$$

4.3.2 Performance Evaluation Using Bovine Serum Albumin (BSA)

Method selected to determine the concentration of BSA in both reservoirs is the Biuret Method (Gornall *et al.*, 1949). The Biuret reagent was prepared by dissolving 6.0 g sodium potassium tartrate tetrahydrate (NaKC₄H₄O₆·4H₂O) in 500 mL distilled water. 1.5 g of copper sulphate pentahydrate (CuSO₄·5H₂O) was then added and dissolved in the solution. 300 mL of 10 %wt/v sodium hydroxide (NaOH), which was freshly prepared, was added slowly with stirring into the solution. Finally, the solution was diluted to 1 L with distilled water and stirred until homogeneous. The Biuret reagent was stored in a bottle covered with aluminium foil and kept in the refrigerator for subsequent use due to its sensitivity to light.

Next, a standard protein solution was prepared. A 20 mg/mL of BSA stock solution was prepared by dissolving 0.5 g of BSA powder in 25 mL of distilled water. During preparation, vigorously stirring is avoided to prevent foaming. Later, 1 mg/mL BSA testing solution was prepared by adding 19.0 mL distilled water into 1.0 mL of 20 mg/mL BSA stock solution. The concentration range of assay was from 0.1 mg/mL – 1.0 mg/mL. Eleven test tubes were washed, dried and labeled from 0-10. Each tube was adding with 1 mg/mL BSA solution and distilled water according to volume indicated in Table 4.2. The solutions were stirred until homogeneous.

Table 4.2 : Standard BSA solution preparation

Tube No.	0	1	2	3	4	5	6	7	8	9	10
BSA vol., mL	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2	0.0
Water vol., mL	0.0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0

Next, 3 mL of Biuret reagent was added into each tube containing BSA solution or sample. The tubes were incubated in 37°C water bath for 15 minutes. The absorbance of each solution was measured against a blank at a wavelength of 550 nm using UV spectrophotometer (UV-Spec Shimadzu UV-160). Each of the tests was run in triplicate. A standard BSA curve is plotted using the data achieved.

The BSA feed solution in the reservoir is prepared by pipeting out 7.5 mL of BSA stock solution of concentration 20 mg/mL into the 142.5 mL of distilled water. The dialysis process was then performed for 3 ½ hours and sample were collected for test every 30 minutes. In order to ensure reproducibility, the test was carried out 3 times.

4.4 Performing Test Using Blood

Finally, the dialysis membrane with the best molecular sieving property was tested with real human blood to mimic the actual situation during hemodialysis. In fact, human blood is much more complex than all the solutions prepared. This test was carried out *in vitro*, in a laminar flow chamber with the least probability of contaminations.

Human blood was obtained from Hospital Sultanah Aminah, JB in blood pack form with anticoagulant inside. This is to prevent the blood from coagulating during the storing and experiment process. Prior to the experiment, the blood was stored at temperature 2 – 4 °C. The *in vitro* hemodialysis circuit was assembled using the single and multi layer dialysis system as other tests were performed. Before used, all the apparatus were sterilized and rinsed using 70 % v/v alcohol.

The dialysate was prepared according to a typical aqueous dialyzing fluid composition (Leonard and Dedrick, 1968) shown below in Table 4.3. This solution was chosen so as to approximate normal body fluid in diffusible calcium, magnesium, sodium and potassium contents. The acetate serves as a pH buffer, while the glucose is added to achieve the desired osmotic pressure (King, 1971).

Both the blood and dialysate reservoir beakers filled with 200 mL of the respective solutions were maintained at 37 °C. The blood was recirculated at a flow rate of 50 mL/min and the dialysate was recirculated at 100 mL/min due to the limitation of the dialysis cell fabricated. The dialysis experiment was carried out for 3 ½ hours and the blood and dialysate samples were taken from the beaker at 0, 120 and 210 minutes, respectively. The earlier samples were stored at temperature 2 - 4 °C and immediately

Table 4.3 : Dialysate composition

Material	Company Supplied	Amount, g/L
CaCl ₂ .2H ₂ O	Merck Co.	0.18
MgCl ₂ .6H ₂ O	Merck Co.	0.15
Na acetate.3H ₂ O	Merck Co.	4.50
KCl	Merck Co.	0.15
NaCl	Merck Co.	5.80
Glucose	Sigma Inc.	2.00

send to diagnostic using RBC Cell-Diagnostic 3200 (Abbot Inc.) and Hitachi 902 Automatic Analyzer. The assays that were carried out for the samples were a normal checklist for dialysis patients such as red blood cell (RBC), white blood cell (WBC), platelet, urea, uric acid and K⁺.

4.5 Results and Discussion

4.5.1 Urea Clearance Performance

4.5.1.1 Effect of Acetic Acid/PEG Ratios

The results of urea clearance with different membranes produced using different molecular weight polyethylene glycol and various ratio of acetic acid/PEG using single layer dialysis membrane system were shown in Table 4.4. Apparently, increasing of the acetic acid/PEG ratio enhanced the urea clearance efficiency for the dialysis membrane

produced regardless of the type of PEG used. As mentioned earlier, higher ratio of acetic acid/PEG indicates a lower amount of polyethylene glycol in the dope solutions, and this improved the membrane's permeability or flux. This observation is in agreement with the study done by Torrestiana *et al.* (1999), which showed that lower PEG content in the membrane formulation would improve its water flux and lower the lysozyme rejection.

Figure 4.2 depicted the plot of urea clearance in a single layer dialysis membrane system versus the ratio of acetic acid/PEG. It is clearly seen that the urea clearance increase as the acetic acid/PEG ratio goes higher (beyond ratio 14), which indicate that lower amount of PEG was favorable. Urea clearance at the ratio point of 16.5 for any three lines depicted was found to be the highest amongst other ratio points and this showed that the PEG amount needed in the formulation was less than 5 %wt. In fact, this result also being supported by Kim and Lee (2004) in their latest findings of ultrafiltration membranes that at higher amount of low molecular PEG used in membrane preparation, the flux will decrease and the solute rejection will increase.

However, there is a limitation to the reduction of PEG content. Membranes produced with very little PEG (very high acetic acid/PEG ratio) are very brittle and easily damaged. As stated earlier, adding hydrophilic additives, PEG in our case, will promotes the rapid demixing of coagulation during phase inversion process (van de Witte *et al.*, 1996). Rapid demixing would produce a membrane with very thin top layer and a sublayer with a lot of macrovoids. Yet, too much of additives will suppress the macrovoids formation. Here, as the PEG amount decrease, the top layer become very thin and the high number presence of macrovoids lead the membrane to become easily rupture.

Table 4.4 : Results of urea clearance in different ratio of acetic acid/PEG with different molecular weight additives

No.	Ratio acetic acid/PEG	Urea Clearance Results, -%		
		PEG 200	PEG 400	PEG 600
1	4	28.43	23.17	26.43
2	6.5	24.92	20.38	22.71
3	9	26.84	21.64	20.43
4	11.5	30.85	28.47	25.64
5	14	37.36	37.49	28.99
6	16.5	40.37	39.52	35.48

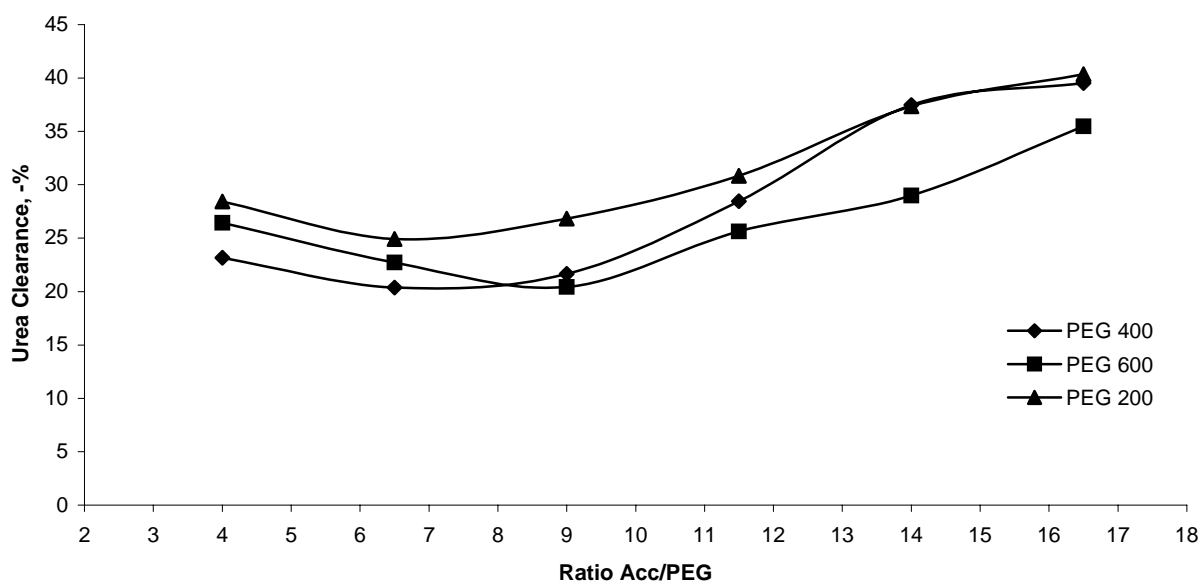


Figure 4.2 Plot of urea clearance at different ratio acetic acid/PEG with different molecular weight additives used

Hayama *et al.* (2004) had carried out another interesting study recently, which showed the role of hydrophilic additives amount in dialysis membrane and the different

resultant biocompatibility caused. According to their study, the membrane surface containing additives would change literally during wet and dry conditions. In terms of swelling, they found that lower amount of hydrophilic additives caused the polymer particle at the surface to swell mainly in the vertical direction or vice versa. The process is illustrated in Figure 4.3. This phenomenon probably contributes to the high urea clearance in our case. With the vertical swelling situation of the polymer on the surface and the spaces created between the polymer structures, the solute passage is made much easier through the membrane. As a result, dialysis membrane with lower amount of additives will encourage the clearance of urea. Yet, this finding is in contra with other works done by several authors, which showed that higher amount of additives encourage the water flux and lower the solute rejection (Khayet *et al.*, 2002; Kim and Lee, 1998).

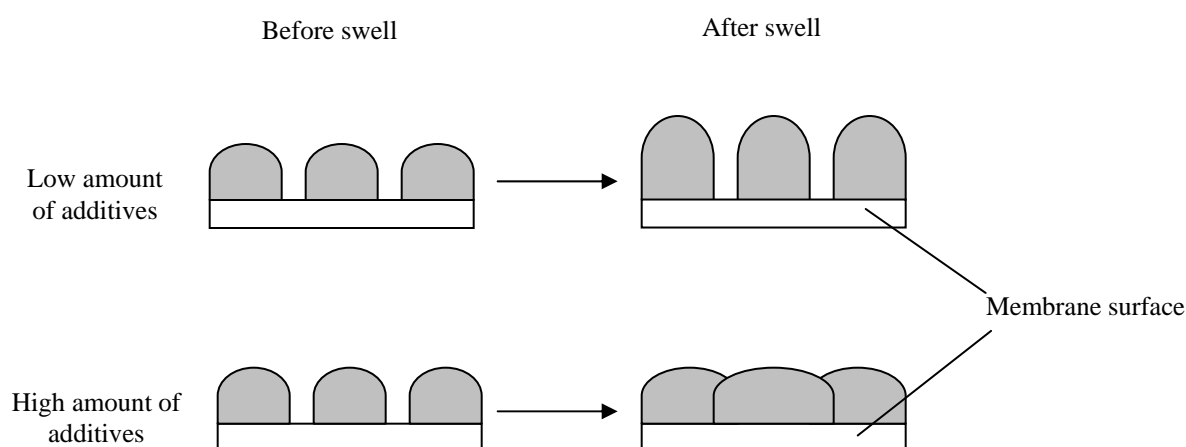


Figure 4.3 Swelling effect with different amount of additives used

Besides, too high amount of additives produced high viscosity dope solutions, which is difficult to cast. Han and Nam (2002) also revealed that the viscosity of dope solution would significantly increase when the amount of additives added beyond 10 %wt and the flux decreased drastically. Furthermore, low non-solvent content also will shorten the deximing time that induced the formation of macrovoids or vice versa. As

mentioned earlier, formation of macrovoids favor the dialysis process that gives the high urea clearance rate. Therefore, it can be concluded that higher ratio of acetic acid/PEG (lower amount of PEG) in dope formulations gives better urea clearance results.

4.5.1.2 Control Experiment

Since decreasing PEG content in the dope formulation will generate better urea clearance performance, a control experiment was carried out to identify the significance of additives in the dialysis membrane produced. From Table 4.4 and the dope formulations, 4 %wt of PEG give best result of urea clearance for any molecular weight additives used. In order to clarify the role of PEG in the membrane, a formulation without PEG is prepared, which only consists of 25 %wt cellulose acetate, 65 %wt acetic acid and 10 %wt water. However, the high amount of solvent in a casting solution is too dilute results the membrane produced will be very brittle and easily damage. In order to avoid this, higher amount of polymer were used in the formulation.

The performance of this membrane was compared to the earlier membrane produced and these results were tabulated in Table 4.5. It was found that the urea clearance for membrane X₂, which is without additives, is much lower than the other dialysis membranes produced. This shows that although only a small of PEG amounts is required, its absence will give a negative impact, which reduce the urea clearance performance of the membrane. This result showed that additional of hydrophilic agent in the dope formulation improves the dialysis membrane performance and this seems to be in agreement with many authors (Hayama *et al.*, 2004; Kim and Lee, 2004; Seong *et al.*, 2004).

Table 4.5 : Urea clearance comparison of control membrane X₂ with other dialysis membrane prepared at ratio 16.5 with different molecular weight additives used

Dope formulation for control experiment, X ₂		Urea clearance for each dialysis membrane produced			
	Content, %wt	X ₂ without PEG	M6, PEG 200	M6, PEG 400	M6, PEG 600
Cellulose acetate	25	20.84	40.37	39.52	35.48
Water	10				
Ratio acetic acid/PEG	-				
Acetic acid	65				
PEG	-				

As mentioned in previous chapter section 3.4.1, the role of PEG as the non-solvent will encourage the mechanism of phase inversion transit from delayed demixing to instantaneous demixing, consequently promotes the finger-like structure that believe in somehow contribute to solute passing through the membrane. Figure 4.4 shows the cross-section image of membrane X₂ without the presence of hydrophilic agents, PEG. It exhibits a dense uniform structure without any macrovoids. The addition of suitable amount of PEG enhances the macrovoids formation as discussed earlier. The absence of PEG in the dialysis membrane promotes a dense spongy non-void structure. Seong *et al.* (2004) also showed that the absence of hydrophilic agents in certain polymer/solvent system would give similar structure and result.

According to Kesting (1985), the role of additives serves to increase the membrane water content (degree of swelling) of the membranes. A positive correlation between water content and permeability was found to exist. Arthanareeswaran (2004)

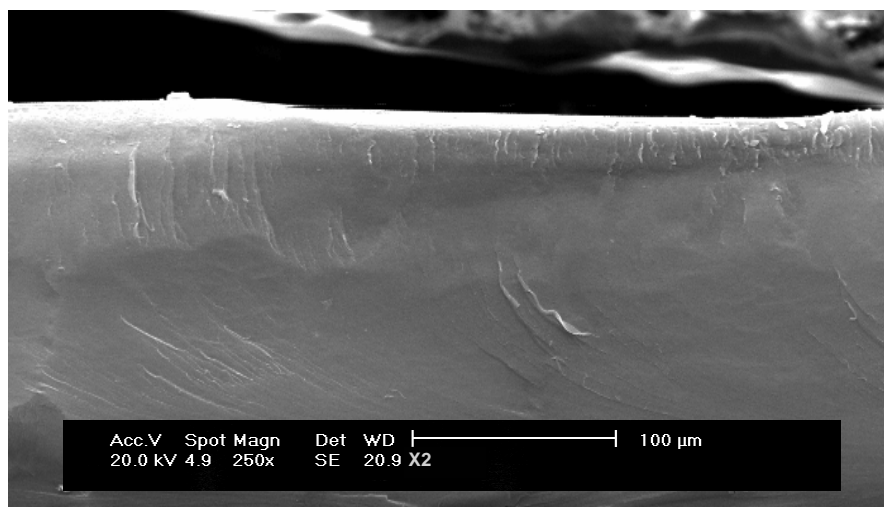


Figure 4.4 SEM cross section image of membrane X₂; without PEG in dope formulation

also reported that addition of hydrophilic additives (PEG 600) play a key role in changing the characteristics of cellulose acetate membrane in improving the permeability of proteins. In our case, the addition of PEG in the dope formulations certainly enhances the urea clearance performance. In addition, hydrophilization with ethylene glycol oligomers is most effective in resisting protein adsorption that will activate the coagulation and complements system during hemodialysis (Prime and Whitesides, 1991). As stated earlier, the formation of macrovoids was essential in the dialysis membranes as it improves the urea clearance performances. The nonexistence of the voids in membrane X₂ definitely does not favor the membrane performance in our case.

4.5.1.3 Effect of PEG Molecular Weight

Results in Table 4.4 showed that membrane produced from lower molecular weight PEG gives better urea clearance percentage. Figure 4.5 shows the normalized

concentration of urea versus time for the 3 different membranes produced using PEG 200, 400 and 600 with the given ratio range. It can be clearly seen that dialysis membrane with PEG 200 are able to remove more urea compared to other membranes, indicated by the steep gradient at 0.0023 ($R = 14$) and 0.0018 ($R = 9$). In contrast, dialysis membrane produced using PEG 600 gives the lowest urea reduction rate, indicated as 0.0014 ($R = 14$) and 0.0011 ($R = 9$). Thus, lower molecular weight additives are more favorable in urea removal with higher rate of reduction.

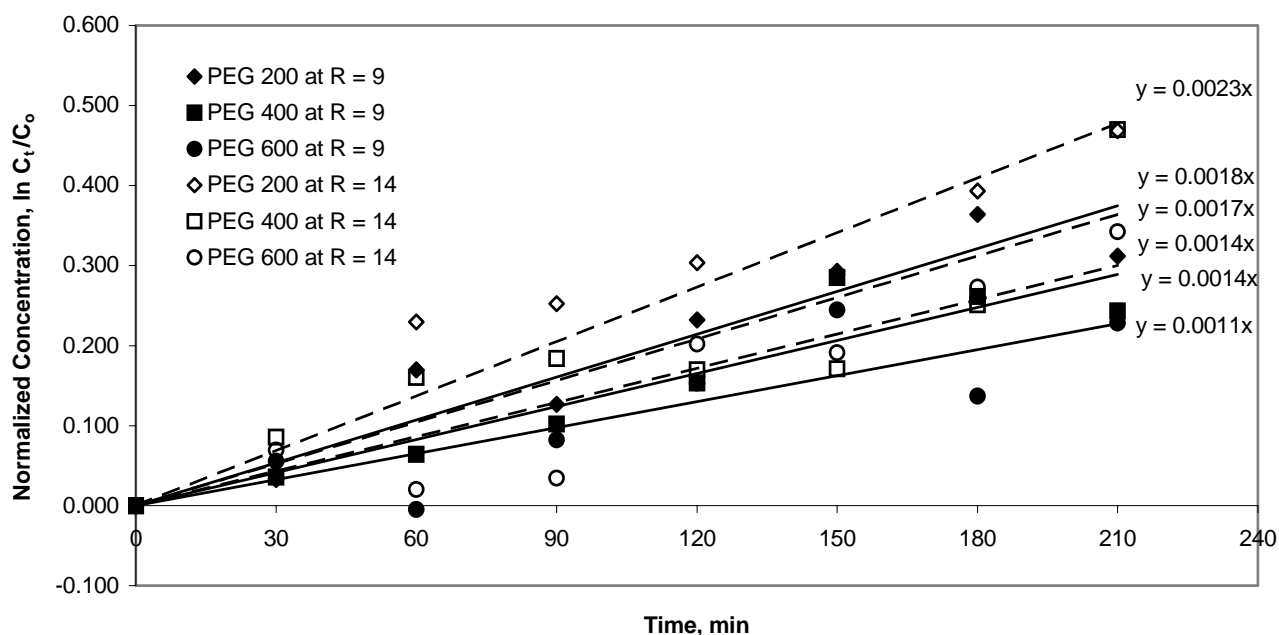


Figure 4.5 Normalized urea concentration as a function of time

The permeability and clearance coefficient of urea of each dialysis membrane with the given ratio and different molecular additives were tabulated in Table 4.6. It was clearly seen that dialysis membrane with PEG 200 as the additives exhibits the highest urea permeability and clearance rate while PEG 600 shows the lowest after 3 ½ hour's period. The membrane permeability using lower molecular weight additives (PEG 200) at the given ratio increased 36 % compared to the higher molecular weight additives used (PEG 600). Furthermore, Table 4.6 also showed that dialysis membrane with PEG 200 as additives enhanced the solute clearance capability as compared to PEG 600 while

Table 4.6 : Permeability and clearance coefficient of dialysis membrane produced using different molecular weight additives at the given ratio

Dialysis membrane	Permeability (x1000), m/s		Clearance, mL/min	
	R = 9	R = 14	R = 9	R = 14
PEG 200	0.124	0.186	15.923	19.366
PEG 400	0.097	0.187	12.893	15.190
PEG 600	0.091	0.136	10.444	12.893

clearance data of membrane with PEG 400 lies between PEG 600 and PEG 200. This can be concluded that dialysis membranes with lower molecular weight additives are favorable in terms of solute clearance and permeability. The effect of the different additives can be explained with the aid of SEM images shown in Figure 4.6 and 4.7.

The effect of the different molecular weight PEG on membrane morphology is clearly observed in Figure 4.6. The macrovoids formation is somehow reduced when higher molecular weight, PEG 600 is used. The rounded shape macrovoids becomes elongated and less sharp with higher molecular weight PEG. The asymmetric thickness becomes more distinct and thicker. This explained for the reduced solute permeability through the dialysis membrane with PEG 600. The thick asymmetric layer create a high resistance to the permeability of the solute permeability and also the flux.

The mechanism of asymmetric membrane formation based on the ratio of non-solvent inflow to solvent outflow was reported (Young *et al.*, 1991). Based on the theory explained, a denser top layer will be formed when the solvent from the casted film rapidly flow out into the coagulation bath. Chuang *et al.* (2000) found that when the casting and coagulant medium came into contact with one another, there is a rapid outflow of the solvent from the casting solution into the coagulation bath thus causing

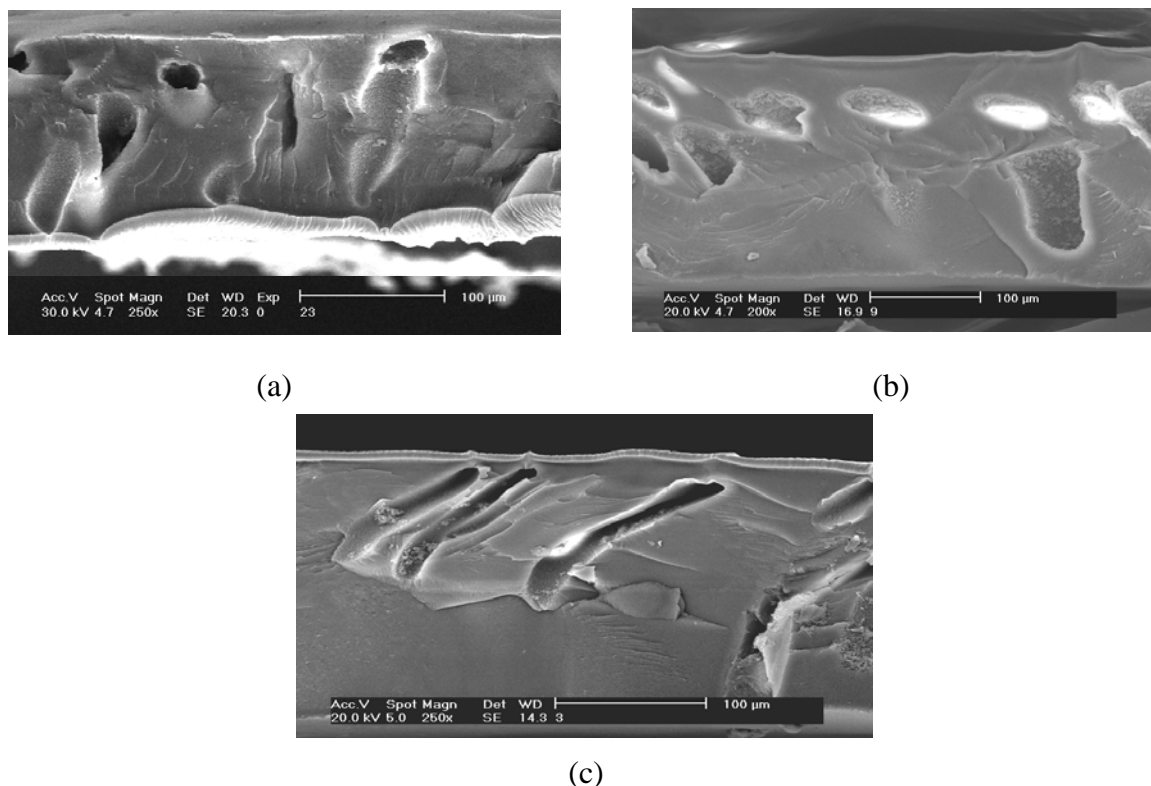


Figure 4.6 SEM cross section image of different PEG used at the ratio of 9, (a) PEG 200, (b) PEG 400 and (c) PEG 600

the higher concentration polymer molecules to aggregate. The presence of different molecular weights PEG effects the formation of the asymmetric layer due to their different diffusion rates. The polymer solution consisting of the higher molecular weight additives (PEG 600) has low diffusion rate and thus promotes the formation of the thick dense asymmetric layer. This same view is being shared by Jung *et al.* (2004).

According to Jung *et al.* (2004), low molecular weight additives is highly soluble than higher molecular weight additives and therefore, it can be washed out together with the solvent from the membrane film to the coagulation bath or vice versa. The rates of diffusivity of higher molecular additives are much slower than the solvent. Therefore, the higher molecular weight additives in solvent take more time to reach the surface and this will give ample time for the polymer aggregates on top of it to form a thicker and denser layer. The top layer become denser with the slow coagulation process and the

macrovoid will be suppressed (Kesting, 1985). By far, most of the dialysis membrane produced using high molecular weight additives consist of thick dense top layer. The dense structure of the membrane increase the rejection of the solute and thus the urea clearance percentage is reduced compared to the membranes prepared using lower molecular weight additives. This result also seems to be in agreement with the study by Yuan *et al.* (2001) that increasing the PEG molecular weight will reduce the surface roughness, macrovoids formation and the permeability of cellulose acetate membranes.

As mentioned in previous chapter, finger-like macrovoids formation favors the urea clearance performance for dialysis membrane. Kesting (1964) reported that the diminishing of the internodular void space would lead to an increase in separation, in other words, the solute rejection increase. Figure 4.7 shows the existence of the finger-like structure in all the 3 membranes produced at ratio of acetic acid/PEG 14. However, as the molecular weight of PEG is increased from 200 to 600, the formation of macrovoids become suppressed and become smaller. The tunnel like structure macrovoids exist almost throughout the entire membrane thickness for the dialysis membrane prepared using PEG 200. As the molecular weight of the additives increase, the tunnel like structure exists only half way through the membrane. The highly interlink of the finger like structure macrovoids in the entire membrane shown in Figure 4.7 (a) assist the urea solute to pass through the membrane. This observation seems to be in agreement with Jung *et al.* (2004) and Yuan *et al.* (2001) that lower molecular weight of additives will enhance the permeability of the solutes and improve the membrane performance.

4.5.2 Comparison of Single Layer and Multi Layer Membrane System

A multi layer membrane system had been applied to improve the urea clearance percentage in single layer membrane system. The background theory was to increase the

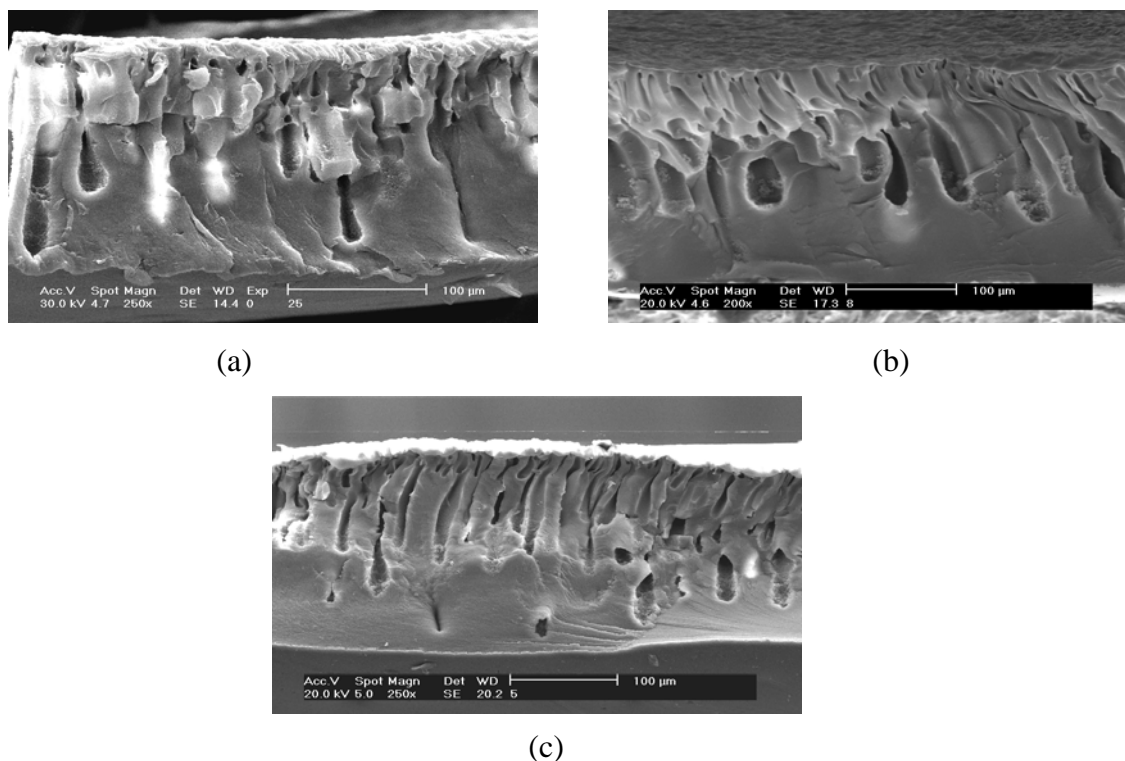


Figure 4.7 SEM cross section image of different PEG used at the ratio of 14, (a) PEG 200, (b) PEG 400 and (c) PEG 600

membrane contacting area of the dialysis membrane from 30 cm^2 to 90 cm^2 . The correlation between the results achieved in single layer membrane and the multi layer were determined to obtain an empirical prediction equation. The results achieved using multi layer dialysis cell are depicted in Table 4.7. Figure 4.8 shows the improvement of the urea clearance percentage compared to single layer membrane system.

It appears the urea clearance increase by 25 – 50 %, regardless of the acetic acid/PEG ratio, when the multi layer unit is used. Generally, increasing the contact area by three fold shift the urea clearance percentage from the 20 – 30 % region to the 30 – 40 % region or even higher as clearly seen in Figure 4.8. There is no doubt that increasing the membrane area will improve the membrane performance. Locatelli and Manzoni (2000) also stated that in order to shorten dialysis treatment time, a larger dialyser surface is required so as to reach adequate solute and fluid removal.

Table 4.7 : Results achieved using multi layer dialysis membrane system as compared to single layer membrane system

No	Ratio acetic acid/PEG	PEG 200		PEG 400		PEG 600	
		Single	Multi	Single	Multi	Single	Multi
1	4	28.43	34.45	23.17	36.69	26.43	43.00
2	6.5	24.92	32.13	20.38	34.79	22.71	41.37
3	9	26.84	31.31	21.64	32.74	20.43	38.37
4	11.5	30.85	37.31	28.47	38.74	25.64	33.14
5	14	37.36	45.57	37.49	40.87	28.99	40.85
6	16.5	40.37	49.54	39.52	46.57	35.48	43.63

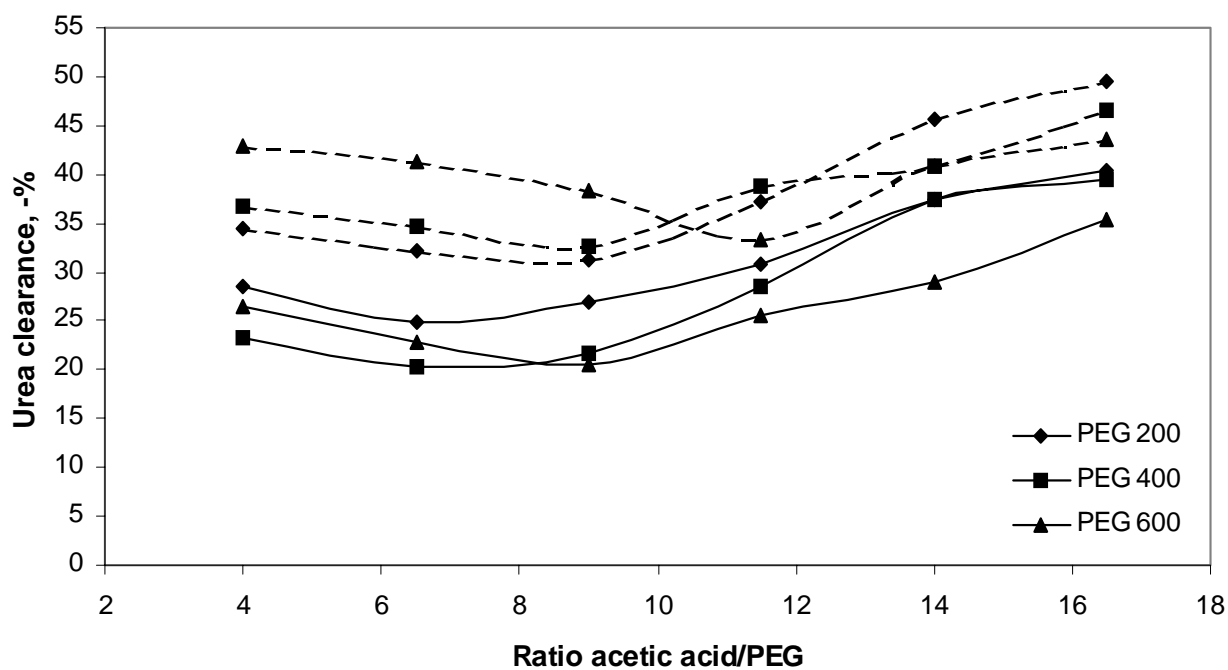


Figure 4.8 Comparison of urea clearance percentage with single layer (—) and multi layer (----) membrane system at different ratio

4.5.2.1 Relationship Between Multi and Single Layer Membrane System

The relationship between a multi and single layer membrane system in our study was basically generated by usual numerical and statistical methods. The hypothesis used for the developing the equation was based on the graphical observation in Figure 4.8. The calculation of the correlations was done by using Microsoft Excel® that determined the function of urea clearance with different system in equation manners. The details of the calculations are shown in Appendix C2.

Since the urea clearance percentage using multi layer membrane was basically shifted up within the same acetic acid/PEG ratio given, the hypothesis here was that the relationship between single and multi layer was a proportional function. The function of urea clearance performance of each polyethylene glycol used, i.e. PEG 200, PEG 400 and PEG 600 was first determined by plot of the urea clearance in single layer membrane system to the multi layer membrane system. The results for both systems were linked and it was found that the correlation between the single and multi layer system all were a second order polynomial derivation. There are three equations generated with the different molecular weight additives used; with each of them having a specific coefficient. The errors of the predicted value and the actual value were less than 5 %, as shown in Table 4.8. The three equations for the different PEG used are shown below.

Additives PEG 200

$$Y = 0.0252 x^2 - 0.4429 x + 26.542 \quad (4.3)$$

Additives PEG 400

$$Y = 0.0083 x^2 + 0.0519 x + 29.726 \quad (4.4)$$

Additives PEG 600

$$Y = 0.0272 x^2 - 1.2079 x + 52.333 \quad (4.5)$$

where Y indicates the urea clearance in the multi layer membrane system and x indicates the urea clearance in the single layer membrane system.

According to Figure 4.8, the urea clearance trend along the acetic acid/PEG ratio for the multi layer membrane system is similar to the results achieved in single layer membrane with any molecular weight additives used. Therefore, second hypothesis were suggested that the coefficient generated by Eq. 4.3 – Eq. 4.5 can be replaced with each other and the three equations can be simplified to one empirical equation that can represent the increment of the urea clearance from single layer membrane system to multi layer membrane system, suitable for PEG 200, PEG 400 and PEG 600. The mean values of those coefficients were calculated and rounded off to 4 decimal places. The equations are shown in Eq. 4.6 and 4.7.

$$Y = 0.020233 x^2 - 0.532967 x + 36.20033 \quad (4.6)$$

$$Y = 0.0202 x^2 - 0.5330 x + 36.2003 \quad (4.7)$$

Table 4.8 : Comparison the actual and predicted results in multi layer membrane system

Ratio	Actual Results			Predicted value at M.			Errors, %		
	PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600
4	34.45	36.69	43.00	34.32	35.38	39.41	-0.38	-3.69	-9.11
6.5	32.13	34.79	41.37	31.15	34.23	38.93	-3.13	-1.63	-6.27
9	31.31	32.74	38.37	32.81	34.74	39.01	4.57	5.75	1.64
11.5	37.31	38.74	33.14	36.86	37.93	39.24	-1.22	-2.13	14.87
14	45.57	40.87	40.85	45.17	43.34	40.18	-0.89	5.69	-1.68
16.5	49.54	46.57	43.63	49.73	44.74	43.72	0.39	-4.09	0.20

In order to associate the membrane contact area as the multiplying factor to the correlation of multi layer to single layer membrane system, the ratio of larger multilayer membrane contacting area to the single layer membrane are taken out from the eq. 4.7 as a constant value. As mentioned in previous chapter, the contacting membrane area for the single layer and multi layer dialysis cell were 30 cm² and 90 cm², respectively. However, the subtraction of constant value from higher polynomial should be avoided. Thus, the final empirical equation developed that relates the urea clearance using single and multi layer dialysis cell are given in eq. 4.9 and eq. 4.10.

$$\text{Ratio of the membrane contacting area} = \frac{A_2}{A_1} \equiv \frac{90 \text{ cm}^2}{30 \text{ cm}^2} \equiv 3 \quad (4.8)$$

$$Y = 0.0202 x^2 - 3 (0.1777) x + 3 (12.0668) \quad (4.9)$$

Urea clearance using multi layer dialysis cell

$$= 0.0202 (\text{Urea clearance in single layer})^2 - 0.1777 (A_2/A_1) (\text{Urea clearance in single layer}) + 12.0668 (A_2/A_1) \quad (4.10)$$

The empirical equation is only appropriate within the acetic acid/PEG ratio of 4 – 16.5 and the given molecular weight additives i.e. PEG 200, PEG 400, PEG 600 in cellulose acetate dialysis membrane produced. In order to validate this equation, the calculated values obtained using eq. 4.10 are compared with the experimental results and the calculated value was tabulated in Table 4.9. It was found that almost the calculated values were in the range of ± 10 % errors. Only 20 % of the data was out of the range. This empirical equation developed was significant in predicting the urea clearance using multi layer dialysis cell.

Table 4.9 : Comparison the actual and predicted results in multi layer membrane system using average coefficient

Ratio	Actual Results			Predicted value at M.			Errors, %		
	PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600
4	34.45	36.69	43.00	37.40	34.71	36.25	-8.57	5.39	15.70
6.5	32.13	34.79	41.37	35.48	33.74	34.53	-10.44	3.01	16.53
9	31.31	32.74	38.37	36.47	34.14	33.76	-16.48	-4.28	12.02
11.5	37.31	38.74	33.14	39.01	37.43	35.84	-4.57	3.39	-7.26
14	45.57	40.87	40.85	44.53	44.66	37.75	2.28	-9.27	7.58
16.5	49.54	46.57	43.63	47.66	46.74	42.76	3.80	-0.36	1.99

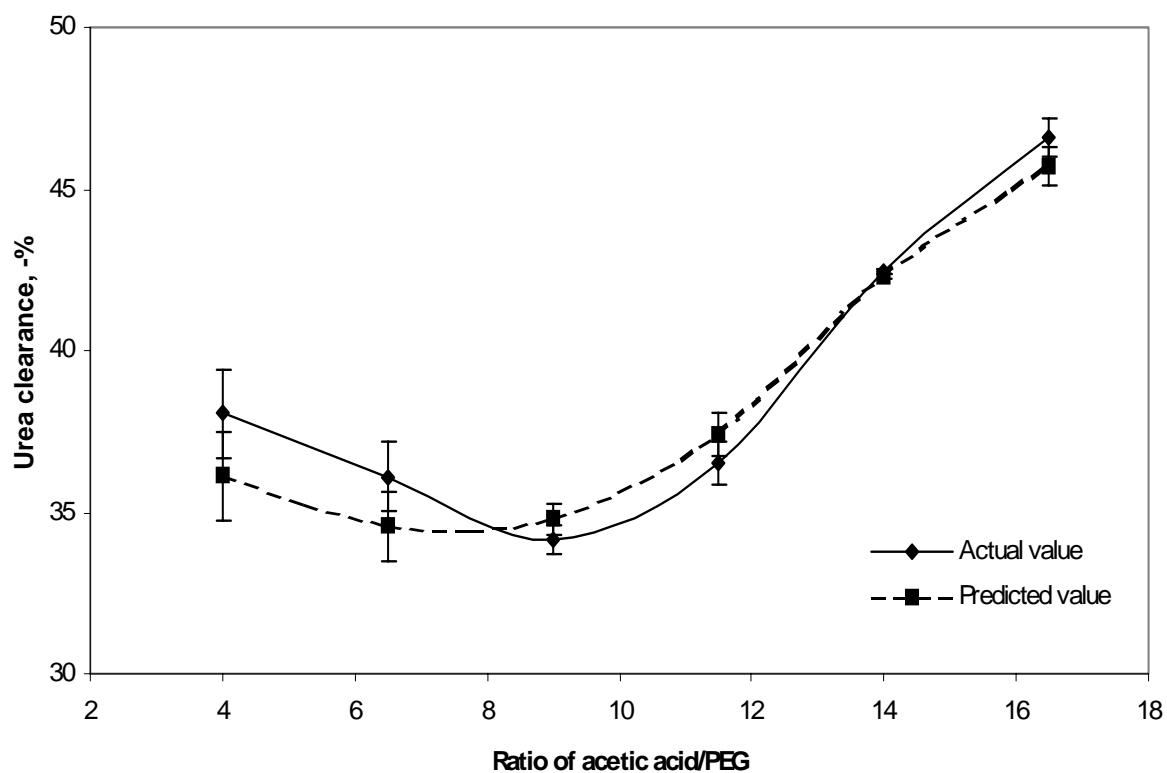


Figure 4.9 Fitness comparison of predicted and actual value

4.5.3 Different Molecular Weight Solute Sieving Efficiency Results

As mention in section 4.3, dialysis membrane is use to removes toxins from human blood but to prevent any human blood protein loss during the process. Therefore, dialysis membrane should have a very specific pore size range. In order to determine the molecular weight sieving efficiency of the dialysis membrane produced using different molecular weight additives, three best membranes were chosen for the solute sieving efficiency test. In addition, this test also differentiates the clearance efficiency cut off of each dialysis membrane produced. The three membranes chosen were dialysis membrane using PEG 200, PEG 400 and PEG 600 as additives at the acetic acid/PEG ratio of 16.5, since these membranes has the best urea clearance performance amongst their groups.

Table 4.10 shows the clearance properties and permeability of each testing solutes calculated using Eq 2.16 and 2.17 and the graphical illustration was depicted in Figure 4.10. It was found that dialysis membrane produced with different molecular weight additives exhibit different molecular clearance efficiency. The dialysis membrane with PEG 200 as the additives shows the highest solute clearance efficiency, followed by membrane with PEG 400 and membrane with PEG 600.

Table 4.10 : Solute clearance efficiency of different dialysis membrane produced

Solutes	MW, kDa	Clearance percentage, -%			Permeability, m/s			Clearance K, ml/min		
		PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600	PEG 200	PEG 400	PEG 600
Urea	0.060	40.37	39.52	35.48	0.205	0.199	0.174	18.033	17.345	14.440
PEG 600	0.600	37.60	30.30	24.37	0.187	0.143	0.111	20.644	12.094	12.094
PEG 3,000	3.000	24.97	16.58	17.43	0.114	0.072	0.076	12.094	8.722	7.834
PEG 10,000	10.000	16.78	11.75	10.21	0.073	0.050	0.043	5.999	5.999	5.051
PEG 35,000	35.000	9.46	7.08	9.21	0.039	0.029	0.038	4.084	4.084	5.051
BSA	66.000	5.17	2.87	1.71	0.021	0.012	0.007	3.095	2.085	2.085

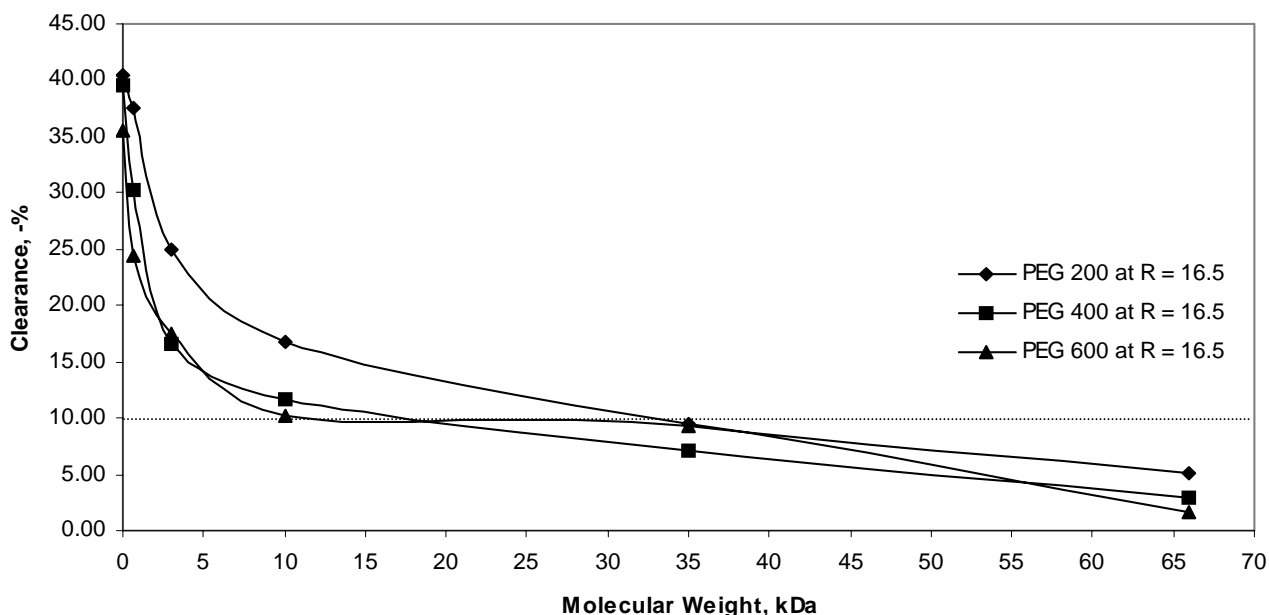


Figure 4.10 Solute clearance efficiency of different additives used in dialysis membrane

Here, we assumed that the solutes clearance lower than 10 % corresponds to the solutes rejection of 90 %. Thus, an estimation of solute clearance cut off range for each dialysis membrane produced can be made. It was found that dialysis membrane with lower molecular weight additives has the highest clearance cut off range and increasing the additives molecular weight will decrease it. From Figure 4.10, the clearance cut off range of the dialysis membrane using PEG 200 is around 33 kDa while membrane with PEG 400 is around 17 kDa and lastly membrane with PEG 600 is around 10 kDa. This explains that cellulose acetate dialysis membrane using lower molecular weight additives will enhance the solute permeability and increase the clearance efficiency.

Figure 4.11 shows the SEM cross-section image of the dialysis membrane produced at the ratio of 16.5. It is observed that all three membranes consists of finger-like structure macrovoids, due to the instantaneous demixing with the additional of low amount of hydrophilic additives. Three different dialysis membranes here have the same

ratio of acetic acid/PEG and therefore, exhibit the similar structure. However, the size and sharpness of macrovoids were different. Under the same magnification, 250x, the dialysis membrane that with lower molecular weight additives (PEG 200) has longer, bigger macrovoids compared to the others dialysis membranes prepared. In addition, the macrovoids exists throughout almost the entire membrane thickness, which extends from the surface to the underneath layer. In contrast, macrovoids in dialysis membrane prepared using PEG 600 as the additives become smaller and only occupy half of the membrane thickness. Generally, high molecular weight additives produce short, less sharp macrovoids structure, diminishing in length.

Macrovoids are quite often observed in the asymmetric membranes made by phase inversion process. They can be favorable for membranes by giving generally higher flux but not for the high-pressure operations (Seong *et al.* 2004). Apparently in dialysis membranes, the presence of macrovoids is favorable with increased solute clearance efficiency.

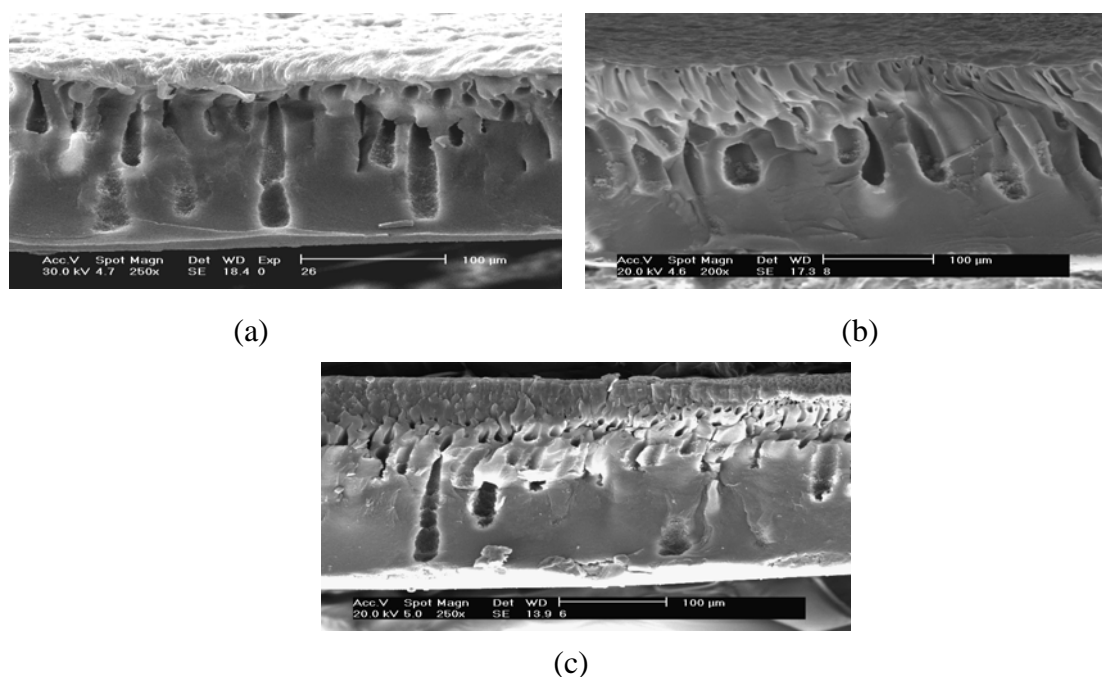


Figure 4.11 SEM cross section image of dialysis membrane at the ratio of 16.5 with different molecular weight additives used, (a) PEG 200, (b) PEG 400 and (c) PEG 600

4.5.4 Blood Test Results

Since membrane 6, which consists of PEG 200 exhibits the best overall performance, it was chosen for the blood test. The final stage dialysis process was carried out using donated human blood stored in blood packs in the single and multi layer dialysis cell. The main purpose of this test is to mimic the actual hemodialysis separation process since human blood is much more complex than any solution prepared in laboratory. It must be noted that the blood inside a blood pack had been pretreated by numerous nutrients solutions such as anticoagulant, dextrose and adsol. Therefore, the concentration of the blood as well as the concentration of the substances appeared in the blood was out of the range of the normal human blood. The main objective of this test was to study the clearance ability of the dialysis membrane in removing certain toxic i.e. urea in human blood without any side effects. Therefore, the results were focused on the depletion of the urea solutes concentration before and after the dialysis process.

4.5.4.1 Blood Test in Single Layer Dialysis Unit

The results achieved after 3-½ hours dialysis process using single layer dialysis cell were tabulated in Table 4.11. The results were fascinating that the dialysis membrane produced can remove up to 24.62 % of urea and 15.95 % uric acid, which are the identified toxic material to human body. The amount of creatinine was also reduced but at lower level due to the higher molecular weight of it compare to urea and uric acid. A well-developed cross-country study work by Ward *et al.* (1997) using different types commercial dialyzers with different type of dialysis membrane, generally have 1.3 m² membrane contacting area, the reduction percentage of urea were 63.0 - 64.6 ± 6.0 %. A group from Belgium (Gerrit *et al.*, 2000) who had also done a similar study with cellulose triacetate membrane contacting area around 1.3 m² – 1.5 m², reported that the

Table 4.11 : Clearance percentage of different substances in human blood by using single layer dialysis cell

Substances	Unit	0 min	120 min	240 min	Clearance percentage, -%
Urea	mmol/l	6.5	6.0	4.9	24.62
Uric Acid	mmol/l	326	308	274	15.95
Creatinine	mmol/l	87	74	82	5.75
WBC	k/ μ l	0.094	0.080	0.081	13.82
RBC	M/ μ l	0.315	0.311	0.297	5.71
Platelet	k/ μ l	11.9	11.3	12.5	-5.04

urea was reduced 75.0 to 77.0 ± 6.1 % after 4 hours dialysis process. These results show that the dialysis membrane produced in this study, with the formulation given above, in such a small scale (30 cm²) is capable to separate uremic toxic from human blood are highly comparable to the current dialysis membrane found.

Moreover, the white blood cell (WBC), red blood cell (RBC) and platelet count (PLT) shows no significant change during the pre and post dialysis. As mentioned by other authors (Krieter and Canaud, 2003), the dialysis membrane produced should be avoid of albumin lost so as to prevent albumin lost associate syndrome such as hypoalbuminaemia during hemodialysis process. The dialysis membrane produced in this study is very reliable, as valuable human blood protein mentioned is not lost during the dialysis process.

4.5.4.2 Blood Test in Multi Layer Dialysis Unit

Since increment of membrane contacting area can increase the solute permeation, the human blood test run was also conducted using multi layer dialysis cell. The optimization equation (Eq. 4.10) developed using statistical and numerical methods was used to predict the urea clearance percentage in human blood test. The results achieved and the predicted value for certain solutes such as urea and uric acid were showed in Table 4.12.

Table 4.12 : Clearance percentage of different substances in human blood by using multi layer dialysis cell and the predicted value

Substances	Unit	0 min	120 min	240 min	Clearance percentage, -%	Predicted value
Urea	mmol/l	8.6	6.7	6.8	20.93	35.32
Uric Acid	mmol/l	279	260	249	10.75	
Creatinine	mmol/l	121	106	106	12.40	
WBC	k/ μ l	1.98	1.73	2.04	-3.03	
RBC	M/ μ l	THD*	THD*	7.91	NA**	
Platelet	k/ μ l	322	310	814	NA**	

* THD = too high for diagnosis (the amount is exceed the maximum diagnosis range)

** NA = data not available or calculation is out of the discussion barrier

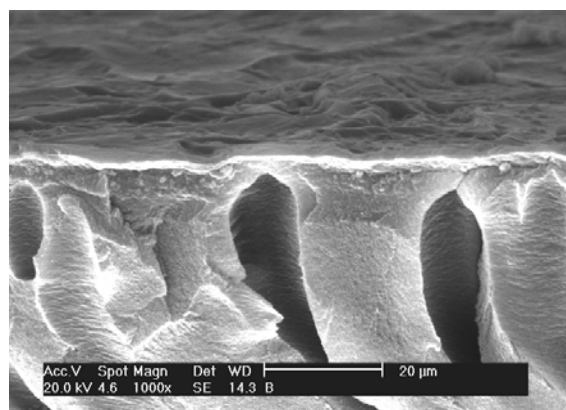
The results obtained by using multi layer dialysis membrane show a pro and cons situation. The clearance of uremic toxic such as urea and uric acid were lower than the results achieved in single layer dialysis membrane. In addition, the values were far lower than the predicted ones. On the other hand, the clearance of creatinine was increased efficiently. Also, it is observed that the amount of human protein, such as white blood cell and red blood cell were too much in the sense of cell count during this dialysis

process. All these most probably caused by there are other transport mechanism held during dialysis process when the membrane come contact to natural human blood complex.

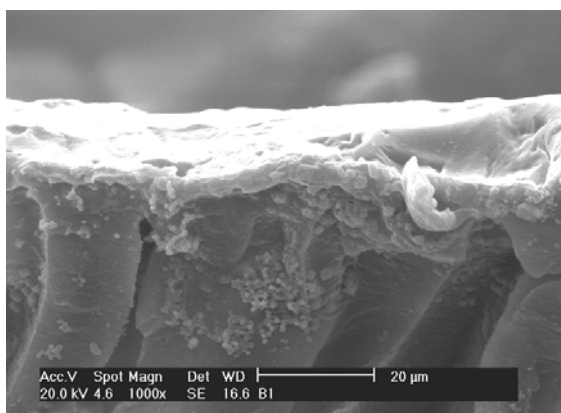
Human complex proteins naturally bind and adsorb to artificial vessel and cause coagulation during dialysis treatment (Valette *et al.* 1999). The presence of high amount human protein in our case will bind on the surface and clog the pores of the dialysis membrane, which will decrease the clearance efficiency. During the dialysis process, it was found that the reduction of bigger molecules such as urea and creatinine stopped around 120 minutes while smaller molecules like uric acid continue to diffuse but at slower rate. This phenomenon might be due to the concentration polarization taking place, where the cell proteins had created another barrier that reduce the diffusion rate of those uremic solutes. Locatelli and Manzoni (2000) also stated that the membrane performance also rely on the distribution of blood in the dialyser and the dialyser geometry. This might explain why the clearance performance of uremic toxic in multi layer system was lower than the single layer ones and the difference between the predicted values using equations and the actual values. In order to study this effect, SEM images were taken before and after the dialysis process.

SEM image in Figure 4.12 shows the cross section of the dialysis membranes produced before and after the blood test. Both dialysis membranes were taken from the same batch of production. It was clearly seen that after the blood dialysis, the skin layer of the membrane was covered by a thick layer of protein substances as seen in Figure 4.12 (b) and (c). The top layer of the after test dialysis membranes also seems to be non-smooth compared to the membrane (a) before the blood test. This observation can be described as the adsorption of blood compounds on to the surface of the dialysis membrane produced. The presence of high amount of cell proteins most probably causes the concentration polarization to occur.

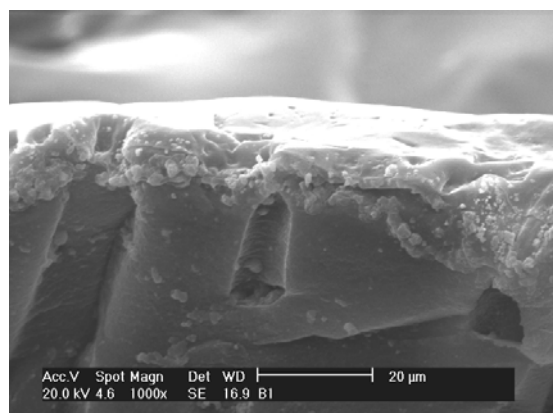
It can also be observed from Figure 4.12 (b) and (c), which were taken from the different portion of the dialysis membrane, that the tunnel like structure in the dialysis membranes were being covered by tiny round shape molecules, which is believed to be the human cell protein and this lowered the solute permeability through the membrane. In contrast, dialysis membrane before the blood separation depicted sharp and smooth macrovoids throughout the membrane. The adsorption of human cell protein on the membrane surface and pores cause the concentration polarization to occur, thus reducing its clearance efficiency.



(a)



(b)



(c)

Figure 4.12 SEM cross section image of dialysis membrane at $R = 16.5$, using PEG 200 as additives; (a) before blood dialysis, (b) and (c) after blood dialysis

Concentration polarization takes place when the solute concentration in the vicinity of the membrane increases until a steady state is reached where the diffusion rate, enhanced by the high concentration near the membrane surface, counterbalances the rate of the solute accumulation near the membrane surface. This phenomenon exerts an unfavorable effect on the performance of membrane separation processes as the high concentration near the membrane surface also increases the osmotic pressure of the feed solution, which results in decreasing in permeation rate (Matsuura, 1993). Aoyagi *et al.* (2004) also stated that protein adsorption on membrane surface that lowers the diffusive permeability should be reduced in order to enhance dialysis adequacy of the patients. This explained that the uremic toxic clearance were decreased with the highly concentration of cell protein in the testing blood. This can be avoided using fresh blood directly from donors that without any pre-treatment instead of using blood from storage pack so as to avoid any coagulation in blood during storage in future study.

4.6 Conclusion

In general, the presence of hydrophilic additives, polyethylene glycol and distilled water in our case are essential in dialysis membrane making process. Overall, the acetic acid/PEG ratio of 16.5 in dope formulation gives the best urea clearance performance regardless of the molecular weight of the PEG additives used. However, in terms of the effect of the different molecular weight PEG to the urea clearance, lower molecular weight PEG would be desired. In our study, membrane with PEG 200 exhibits the highest urea clearance rate. Moreover, the permeability and the clearance rate of dialysis membrane produced using low molecular weight additives (PEG 200) and higher ratio of acetic acid/PEG ($R = 16.5$) shows the highest value.

Increase in membrane contacting area indeed enhanced the urea clearance performance. The increments of the membrane performance using multi layer membrane system were in the range of 25 – 50 % compared to single layer membrane system. The model of optimization generated to predict the urea clearance value in multi layer membrane in the given acetic acid/PEG ratio of 4 to 16.5 was a second order equation. The predicted values were proved to be reasonably accurate as the error between the actual and predicted values is less than 10 %.

In the determination of molecular weight clearance efficiency, it was found that dialysis membrane with low molecular weight additives, PEG 200, gives the highest clearance cut off value. The clearance cut off value, which represent the clearance efficiency of the different dialysis membrane produced using PEG 200, PEG 400 and PEG 600 were 33 kDa, 17 kDa and 10 kDa, respectively.

Finally, it is proven that the best dialysis membrane produced is capable to remove toxin such as urea, uric acid, K^+ as well as middle large molecules such as creatinine from human blood. In fact, the dialysis membrane produced was highly biocompatible that it would not allow human protein to pass through the membrane. However, natural cell protein in human blood such like red blood cell and white blood cell tend to clog and bind to the surface of the dialysis membrane produced and this problem can be reduced using fresh blood directly from the blood donors.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 General Conclusions

This chapter was written with the intention of reviewing the results in order to summarize the work and consolidate the detailed discussions and conclusions that have already been presented at the end of every chapter. Most of the previous works on dialysis membranes had only focus on the effect of the type of dialysis membranes on the health of dialysis patients but seldom investigate the polymer composition in dialysis membrane. Furthermore, most of the research performed use traditional experimental method and the investigations were not done in a coherent manner. By applying the response surface methodology, simultaneous examination of many factors that are thought to influence the membrane performance could be studied with limited experiments.

The response surface methodology, which was used in the first phase of the study, identified that the ratio of acetic acid/PEG is a significant factor that influence cellulose acetate dialysis membrane performances in terms of urea clearance. In contrast, the amount of water in the dope formulations does not give any significant influence to the membrane performance due to PEG in our case play a more dominant role as the non-solvent. But the presence of water in the dope for cellulose acetate dialysis membrane is important as the second non-solvent that enhance the formation of macrovoids consequently promotes urea clearance performance. Dope formulation without water does not give good results for urea clearance. The correlation of urea clearance to the ratio of acetic acid/PEG was proved to be a quadratic model. Suitable low amount of additives, PEG, which is less than 5 %wt, enhance the urea clearance. In addition, increased amounts of PEG produced high viscous dope solutions, which do not favor the membrane casting process.

As mentioned earlier, the ratio of acetic acid/PEG is the significant factor that influences the dialysis membrane performance. Therefore, in the second phase, further experiments involving 6 different ratios (ranging from 4 to 16.5) were performed. It can be concluded that lowering the amount of PEG in the dope formulation promotes the urea clearance. However, dope formulation without PEG does not give good results for urea clearance and there is a limitation to the reduction of PEG content. Membrane with very little PEG (very high acetic acid/PEG ratio) is very brittle and can be easily damaged. The membrane containing acetic acid/PEG of 16.5 is the best formulation for dialysis membrane as it gives the membrane with excellent performance in terms of urea clearance.

Since the hydrophilic additives, PEG is the most significant factor that influences the membrane performance, further investigations were carried out to study the effect of the different types of PEG, namely PEG 200, 400 and 600. The experiment results revealed at the same given ratio, membrane with low molecular weight additives (PEG

200) exhibits best urea clearance performance (40.37 % of removal) compared to the other higher PEG molecular weights used (39.52 % for PEG 400 and 35.48 % for PEG 600). Additionally, the permeability and the clearance rate of the dialysis membranes produced using PEG 200 and high acetic acid/PEG ratio shows excellent performance amongst the other membranes.

SEM cross-section images revealed that at an appropriate amount of the additives would promote the formation of macrovoids in the membrane structure. Increasing the amount of PEG will suppress the macrovoids formation. SEM images also showed that the amount of water in the dope formulation gives a strong influence to the membrane morphology. When the water content is increased, the macrovoids will be suppressed. Additionally, different molecular PEG also gives different membrane structure. The formation of macrovoids exists almost throughout the entire membrane thickness for the dialysis membrane prepared using PEG 200 while as the molecular weight increase; the macrovoids exist only half way through the membranes. Furthermore, increasing the molecular weight of additives will suppress the macrovoids formation.

Increase of membrane contacting area, from 30 cm^2 to 90 cm^2 , enhanced the urea clearance performance. The improvements of the membrane performance using multi layer membrane system were in the range of 25 – 50 % compared to the single layer membrane system. The model of optimization generated to predict the urea clearance value in multi layer membrane in the given acetic acid/PEG ratio of 4 to 16.5 was a second order equation. This model is reasonably accurate with an error of $\pm 10\%$ regardless of the molecular weight of additives used.

The molecular clearance efficiency experiment results revealed that dialysis membrane with PEG 200 as the additives gives the highest clearance cut off value of 33

kDa. The formulation containing PEG 400 and PEG 600, produced membranes with lower molecular cut off clearance values of 17 kDa and 10kDa, respectively.

In the final part of the research, the dialysis membrane with the highest clearance rate was tested with human blood in order to mimic the actual hemodialysis separation process. The dialysis membrane produced using cellulose acetate 20 %wt, acetic acid 66 %wt, polyethylene glycol 4 %wt and distilled water 10 %wt was successful in removing uremic toxic like urea, uric acid, K^+ as well as middle large molecules such as creatinine. In general, the dialysis membrane produced in our study was reasonably biocompatible, as it would not allow human cell protein to pass through the membrane. However, natural cell protein in human blood such like red blood cell and white blood cell tend to clog and bind to the surface of the dialysis membrane produced. This problem can be reduced using fresh blood directly from the blood donors.

5.2 Recommendations for Future Work

Further studies in a number of aspects relates to the present investigation could be carried out in order to comprehend various issues.

- 1) The dope formulation prepared could be spun into hollow fiber modules, which has higher ratio of area/volume value to further study the effect of contacting area.
- 2) Numerous hydrophilic additives such like larger molecular weight polyethylene glycol, polyvinylpyrrolidone (PVP) and acetone can be added in the dialysis membranes and its effects studied.

- 3) Multiple layers composite material coating onto the dialysis membrane can also be fabricated so to improve its selectivity.
- 4) Characterization techniques such as electron spin resonance (ESR), RAMAN spectroscopy (RS) and atomic force microscopy (AFM) could be used to analyze the membrane's morphology.
- 5) Further experiments can also be carried out using various indicators such like C3a and C5a in order to define the biocompatibility of the dialysis membrane produced.